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Review RRJoLS

ISGylation: A Key Host Cellular Defense Mechanism

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Abstract

ISGylation, a crucial process of the innate immune response, has garnered increasing attention for its involvement in host's defense against pathogenic infections as well in cancer progression. The key player of ISGylation, ISG15, is stimulated by type I interferons (IFNs). This5 covalently binds with viral (influenza NS1A and nucleoproteins) and host (Nedd4, filamin B and CHMP5) target proteins and this interaction inhibits the viral replication process and release of viral particles respectively. The ISGylation process dynamically modifies the immune signaling pathways mediated by NFkB, JNK, and IRF-3, which are crucial for immune response. ISG15 has diverse roles beyond ISGylations, for instance the free extracellular ISG15 serves as immunostimulatory cytokine in response to type-I interferon expression and free intracellular ISG15 is involved in stabilization and destabilization of various cellular proteins such as Ubiquitin-specific protease 18 (USP18) and Cyclin D1. The reversal of ISGylation is known as deISGylation where USP18 an ISG15 specific protease plays a crucial role. USP18 is a potent inhibitor of interferon signaling thus inculpated in the suppression of innate immune response in host cell. The future studies focusing on ISGylation research encompass a deeper exploration of its molecular mechanisms, diagnostic utility, crosstalk with other immune responses, and potential therapeutic interventions. This review consolidates updated literature on state-of-art ISGylation and its role in providing immunity to human host together with deISGylation process, and strategies to counter deISGylation. By advancing our comprehension on ISGylation we can more effectively harness ISGylation's potential to strengthen immune function and provide novel therapies for infections and cancer.

Keywords: ISG15, innate immunity, ISGylation, DeISGylation, USP18, pathogenies

INTRODUCTION

ISGylation is a process of post-translational modification that is crucial for the immune system, playing a significant responsibility in defending the host against viruses and beyond [1, 2]. The initial line of defense against viruses and other pathogenic diseases is innate immunity. Ubiquitin cross-reactive protein, or interferon-stimulated gene 15 (ISG15), a protein that is strongly induced by alpha interferons is pivotal in this response [1, 2]. ISG15 is an interferon-induced protein that plays a significant role in the host antiviral response by directly impending viral replication and regulating host immunity [3]. As a cytokine, ISG15 has been documented to engage in a variety of intracellular protein interactions and has been identified in the serum of interferon-treated individuals as well as in mice that are contaminated with viruses [4]. It has been identified that it has many immunomodulatory functions as a cytokine, including influencing IFN signaling, controlling cytokine release, and promoting immune cell maturation [5]. The process of ISGylation entails the peptide linkage of ISG15 to residues of lysine on substrate proteins. A series

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of enzymes, such as ISG15-activating enzyme (E1), ISG15-conjugating enzymes (E2), and ISG15-protein ligases (E3), aids in this mechanism [6, 7]. The enzyme USP18 is capable of deconjugating this modification [8, 9]. Over 100 proteins are ISG15 substrates, underscoring ISGylation's broad cellular functions [2].

The first ubiquitin-like protein discovered was referred to as ISG15, it possesses two ubiquitin-like domains akin to ubiquitin [1, 10]. ISG15 exists as 17 kDa protein and matures to 15 kDa form which can then be covalently bounded to proteins via the enzymatic pathway of ISGylation [2, 11]. The diversity of ISG15's functions is accentuated by the observation that Patients lacking ISG15 exhibit type I interferon autoinflammation without seeming to be more vulnerable to viral infection, indicating functional diversity between species [2]. ISG15 and ISGylation have been associated in a variety of contexts beyond viral infections, including non-viral infectious diseases, cancers, and assorted cellular activity such as the regulation of interferon-responsive cell death [2]. The role of ISG15 in human diseases, including Covid-19, cancer and neurodegenerative disorders, highlights its importance in pathophysiological processes [2]. When ISGylation is eliminated from target proteins, it is referred to as deISGylation activity which is carried out by enzymes called USP18. It involves cleaving the target protein's SUMO protein [12]. Numerous cellular pathways, including immunity, antiviral defense, and cellular stress responses, depend on ISGylation for regulation.

ISGylation has evolved as a versatile and dynamic process within innate immunity. The understanding of ISG15 and the ISGylation mechanism continues to expand, revealing its significance in both health and disease [2].

A DEFENSE MECHANISM FOR HOST-ISGYLATION

In the ongoing battle against viral infections, the host's innate immune system deploys a formidable defence mechanism known as ISGylation. This involves ISG15 conjugation to proteins, playing a pivotal role in controlling the immunological defense and thwarting viral replication. The initiation of ISGylation is triggered by the induction of the ISG15 gene, stimulated by the detection of viral components, primarily facilitated through the alpha interferon signaling pathway [13].

The pathway of ISGylation parallels that of ubiquitination, involving sequential catalytic reactions (Figure 1). The E1 enzyme UBE1L is involved in the cascade's activation and conjugation is facilitated by the E2 enzyme UbcH8, and ligation to substrates facilitated by an E3 ligase such as HERC5 or EFP (Table 1) [14]. Conjugates ISG15 to proteins, termed ISGylation, exerts multifaceted effects on their function, stability, and localization, thereby bolstering the antiviral state of the cell. ISG15 regulate the immunological response by amplifying production of interferons and cytokines [15]. The effects of ISGylation on cellular processes are diverse and complex. Notably, ISGylation can impede viral replication by modifying viral proteins, disrupting their function or stability. Furthermore, it regulates antiviral defense pathways, such as enhancing the production of interferons and cytokines [16]. A remarkable aspect of ISGylation is its reversibility. Specific proteases, such as USP18, mediate the deconjugation of ISG15 from modified proteins. This dynamic regulation calibrates immune response, ensuring that excessive ISGylation, which could disrupt cellular homeostasis, is prevented [17].

Recent advances in structural biology have elucidated ISGylation's molecular mechanisms. The assembly of the UBE1L-UBE2L6-ISG15 complex has revealed intricate details of ISG15 recognition and the recruitment of UBE2L6 by UBE1L [18]. Over 100 proteins identified as ISGylation substrate highlight its wide cellular impact. These substrates impact diverse roles in viral replication, inflammation, cell proliferation, differentiation, and tumor development [19]. Dysregulation of ISGylation has been implicated in a spectrum of disorders, including autoimmune disorders, chronic viral infections, and cancer. Consequently, targeting ISGylation holds immense promise for development of therapeutics for these conditions [2].

Table 1. Enzymes involved in ISGylation activity.

Enzyme Abbreviation	Enzyme Name	Type of Enzyme	Role in ISGylatio
UBE1L	Ubiquitin-like modifier-activating enzyme 7 (UBA7)	E1 activating enzyme	Activates ISG15 by forming a high-energy thioester bond with it, initiating the ISGylation cascade (58).
UBE2L6	Ubiquitin-conjugating enzyme E2 L6 (UbcH8)	E2 conjugating enzyme	Transfers the activated ISG15 from the E1 enzyme to the E3 ligase, facilitating the conjugation step (58).
HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	E3 ligase enzyme	Catalyzes the final step of ISGylation by ligating ISG15 to lysine residues on substrate proteins (58).
TRIM25	Tripartite motif-containing protein 25	E3 ligase enzyme	Functions as an E3 ligase in the ISGylation process, facilitating the transfer of ISG15 to target proteins (58).
EFP	Estrogen-responsive finger protein	E3 ligase enzyme	Acts as an E3 ligase, promoting the ISGylation of specific target protein (58).
UBC13	Ubiquitin-conjugating enzyme E2 N (Ubc13)	E2 conjugating enzyme	May work in conjunction with UBE2L6 to transfer ISG15 to target proteins (58).
UBCH6	Ubiquitin-conjugating enzyme E2 E1 (Ube2E1)	E2 conjugating enzyme	Potentially involved in the ISGylation process, though its specific role is not detailed in the provided key points (58).
UBCH8	Ubiquitin-conjugating enzyme E2 L3 (Ube2L3)	E2 conjugating enzyme	Involved in the ISGylation process, possibly in a similar capacity to UBE2L6 (58).
USP18	Ubiquitin-specific peptidase 18	De-ISGylating enzyme	Removes ISG15 from conjugated proteins, reversing the ISGylation modification (58).

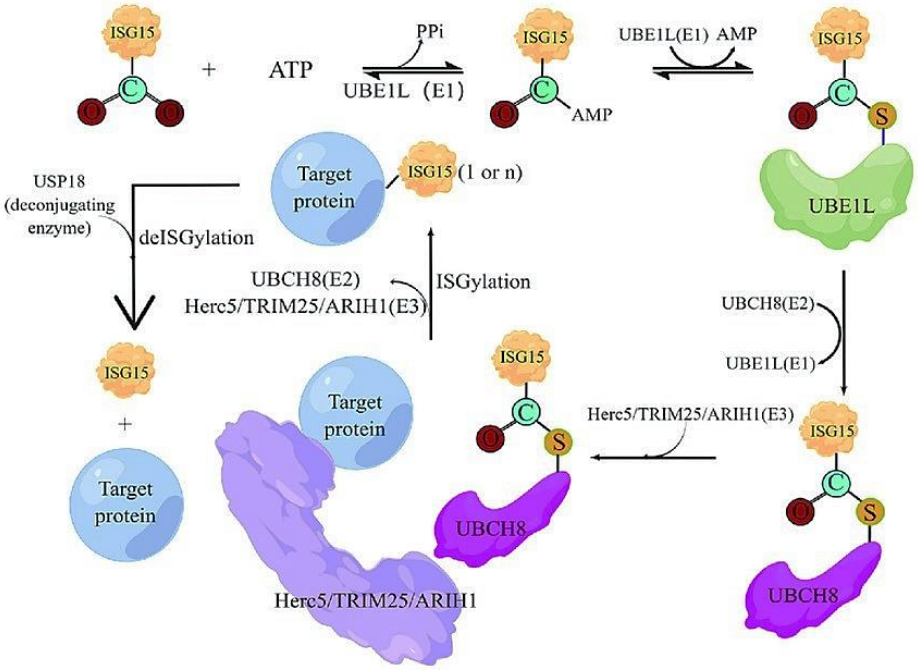

Figure 1. The ISGylation cascade mechanism. Adapted from Yuan Y, Qin H, Li H, Shi W, Bao L, Xu S, et al. The functional roles of ISG15/ISGylation in cancer. Molecules. 2023 Jan 31;28(3):1337 [19].

ISG15-KEY PLAYER OF ISGYLATION

ISG15 Conformation

The 17-kDa protein ISG15 is one of the prominent ISGs produced during pathogenic infection and resulting type I interferon response [20]. The gene encodes initial inactive protein that undergoes cleavage of eight amino acids that C-terminal. This cleavage results in exposure of a crucial motif 'Leu Arg Leu Arg Gly Gly' (LRLRGG) at C-terminal which is requisite for its conjugation with its target proteins [21, 22]. ISG15's protein structure is comprised of two domains: the C-terminal LRLRGG

motif, which is necessary for ISGylation, and the ubiquitin-like domain (Ubl). Like ubiquitin, the ISG15 Ubl domain has a β-grasp fold made up of five β-strands organized in a β-sheet core with an α-helix on either side (Figure 2) [21, 23].

Free ISG15: Intracellular & Extracellular Role

Free Intracellular ISG15

Intracellular ISG15, when unbound to other molecules, can interact with intracellular proteins through non-covalent associations, impacting their functions and interactions [21]. One such interaction involves ISG15 binding to NEDD4, reducing NEDD4's activity and subsequently inhibiting the ubiquitination of VP40. Unbound ISG15 may possess antiviral properties by interfering with the host cell's ability to mark proteins for degradation. Another interaction occurs between ISG15 and USP18, disrupting the interaction between USP18 and SKP2 [21, 24]. This disruption prevents the USP18 degradation by proteases, a process crucial for adverse response regulation of interferon signaling and avoidance of autoimmune inflammation [24]. Overall, free intracellular ISG15 appears to modulate protein-protein interactions (PPIs) within the cell, potentially influencing antiviral responses and immune response [24].

Free Extracellular ISG15

It is widely acknowledged that free extracellular ISG15 has immunomodulatory effects on the body, but there is still much to learn about how it works and its role in the body [21]. Unlike other proteins, ISG15 does not have a signal peptide for secretion, may be released from cells uniquely [21]. It might be stored in certain cell structures, like neutrophil granules and micro-vesicles, before being released. Researchers discovered that ISG15 can be secreted from cells in response to signals, like toll-like receptor 3 activation or cell death. Current research shows, free extracellular ISG15 can bind to a receptor called lymphocyte function-associated antigen 1 (LFA-1) on the exterior of immune cells [25]. Thus, activates proteins called SRC family kinases (SFKs), which then trigger the secretion of other molecules like IFNγ and IL-10, these molecules are crucial to immunological reaction [25]. The form of ISG15 (as dimer or multimer) seems to be important for its ability to act as a cytokine and induce the synthesis of IL-1β, especially during parasite infections [26].

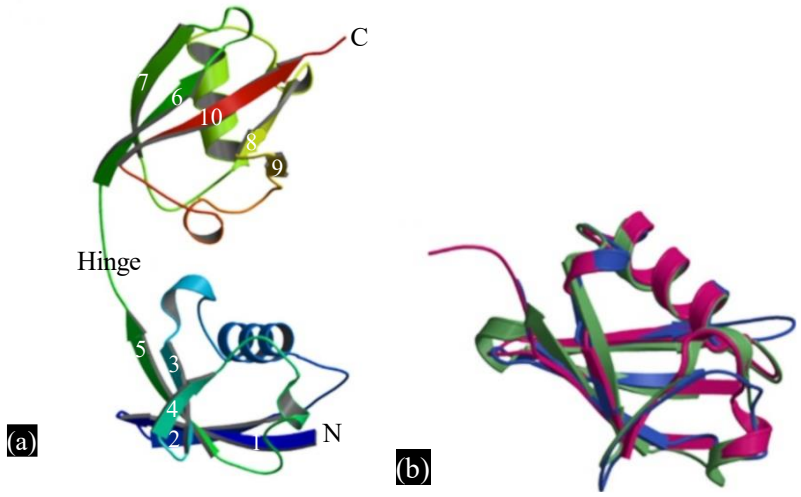

Figure 2. (a) ISG15 ribbon diagram, with colors ramping from blue (N terminus, N) to red (C terminus, C) via green (Hinge), illustrating two distinct domains. The two ubiquitin-like domains (each in the β-grasp fold) are hinged together and have distinct orientations. The disorganized and unresolved state of the final four C-terminal residues of ISG15 demonstrates the flexibility of the C-terminal tail. (b) ISG15 resembles Ubiquitin fold is clear by superimposing the ribbon representations of ubiquitin (pink) with the ISG15 (green) domains. Adapted from Narasimhan J, Wang M, Fu Z, Klein JM, Haas AL, Kim JJP. Crystal structure of the interferon-induced ubiquitin-like protein ISG15. J Biol Chem. 2005 Jul;280(29):27356–65 [23].

Various cell types, including fibroblasts, monocytes, plasmablasts, and neutrophils, can secrete ISG15 in different ways, either in response to type I interferons or independently [26]. ISG15 has been identified in serum of patients undergoing treatment with IFNβ and infected with hepatitis B. As a cytokine, ISG15 can enhance the activity of monocytes, stimulate the production of IFNγ and NK cells, and mature dendritic cells (Figure 3). In monocytes, ISG15 can also boost the secretion of IL-10, which could be an advantageous marker for assessing the acuteness of tuberculosis [15].

Extracellular ISG15 found in the plasmablasts from patients with lupus, although its exact role whether protective or harmful in the disease is not yet clear [27]. Remarkably, research has shown that the absence of extracellular ISG15 (aside from intracellular ISG15) correlates to a decrease in lymphocytes' ability to produce IFNγ and an increase in resistance to mycobacterial infections, underscoring extracellular ISG15's critical role in combating these infections [15].

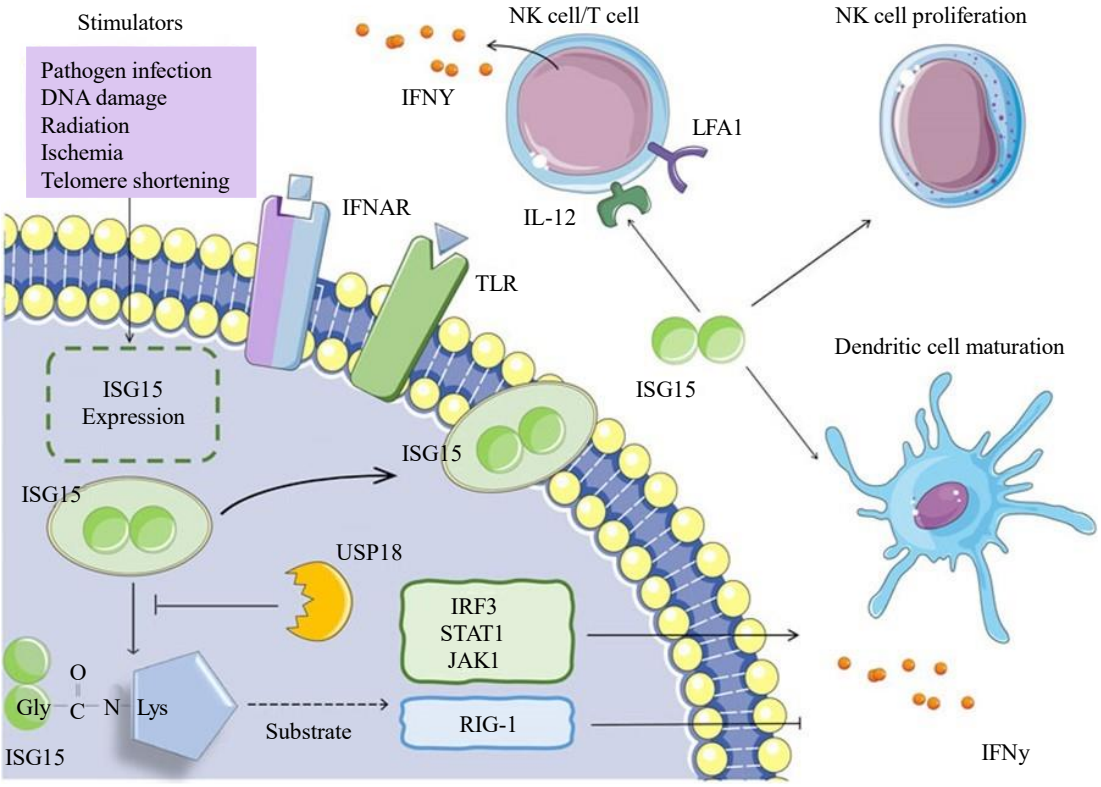

Figure 3. ISG15's role in the immune response. It is likely for monocytes, lymphocytes, neutrophils, and other pathogenic stimuli such as bacterial and viral infections, lipopolysaccharide (LPS) and DNA damage to cause ISG15 secretion. Intracellular ISG15 can attach itself to innate immunity signaling pathway-related proteins, activate IRF3, STAT1, JAK1, and other proteins, or block protein function (like RIG-I), all of which can either increase or decrease IFNγ release. ISG15 produced in vitro can attach to the cell surface LFA1 receptor, stimulating the release of IFNγ from T and NK cells. Additionally, it can stimulate dendritic cell maturation and NK cell proliferation. Adapted from Zhang M, Li J, Yan H, Huang J, Wang F, Liu T, et al. ISGylation in innate antiviral immunity and pathogen defense responses: A review. Front Cell Develop Biol. 2021 Nov 25;9:788410 [63].

ISG15'S DEFENSE MECHANISM AGAINST PATHOGENS & DISEASES

Viral Infections

ISG15 in COVID-19

ISGylation is a key modulator of the immunological response to viral infections, including SARS-CoV-2 and closely related to the pathogenesis and immune response in COVID-19. ISG15 conjugation to target proteins is a crucial step in this mechanism and contributes substantially to antiviral defense

[28, 29]. During SARS-CoV-2 infection, the virus triggers a shift from protein conjugation (ISGylation) to the accumulation of free ISG15 [28]. This shift is driven by the virus's papain-like protease (PLpro) enzyme, which leads to deISGylation, this results in a higher free vs conjugated ISG15 ratio (28). This altered balance is associated to the polarization of macrophages towards pro-inflammatory traits, which might be liable for the cytokine storms detected in acute COVID-19 patients [28]. Additionally, SARS-CoV-2 is capable of bypassing innate antiviral defenses by interfering with the ISG15-dependent MDA5 activation, a pattern recognition receptor, and IRF3, a transcription factor that aids in the defense against viruses [30]. The morbidity and severity of COVID-19 have been associated with the virus's capacity to regulate ISGylation and deISGylation processes, which can result in abnormal immune responses such as chronic inflammation and cytokine storm [30]. The release of extracellular unbounded ISG15 serves as a cytokine that intensifies inflammation during SARS-CoV-2 infection [30]. It also indicates that regulating super-inflammation in COVID-19 patients might require targeting the virus's PLpro or mitigating extracellular ISG15 [30]. The virus's ability to alter the ISGylation pathway contributes to the dysregulation of macrophage responses and the severe inflammatory reactions recognized in COVID-19. Understanding the capacity of ISGylation in COVID-19 pathogenesis opens possibilities for therapeutic interventions aimed at restoring the balance of ISGylation and controlling the regulation of the immune response.

ISG15 in HIV

Various viruses disrupt cellular signaling and antiviral defense by modulating the Ub-proteasome pathway [31, 32]. Ubiquitination is used by HIV-1 at two stages of its replication cycle. First, cellular cytidine deaminase APOBEC3G is targeted by HIV-1-encoded protein Vif for Ub-mediated destruction. Throughout reverse transcription, this prevents APOBEC3G from incorporating viral components and from damaging the viral genome [31, 33, 34]. Second, Ub ligase Nedd4.1 must ubiquitinate Gag for virions to assemble and be released from contaminated cells [31, 35]. HIV-1 accumulation is mediated by the Gag poly protein [31, 36], and the proliferation of HIV-1 virions at the plasma membranes is caused by elimination of Gag's PTAP-L domain from p6 or interference with Gag's association with the endosomal sorting complex protein Tsg101 (ESCRT-I) [31, 37]. The ablation of excessive amount of IFN generating dendritic pDC2 cells resulted in fast development of HIV-1 contamination in vivo, suggesting that type I IFN is a vital component of innate barrier against HIV-1 infection [38]. It has been researched that type I IFN inhibits HIV-1 replication in the last stages of viral assembly and emergence [39, 40], and that the reduction of HIV-1 replication by IFN-α is linked to the upregulation of ISG15 [41]. HIV-1 particles cannot be released from contaminated cells without the Gag polyprotein and the PTAP motif of p6-Gag, and an association has been identified between the ubiquitination of Gag and viral particle exocytosis [42]. The research further showed that IFN's antiviral activity is mimicked by ectopic production of ISG15 and UBEL1, and that IFN inhibition is reversed, and HIV-1 release remains intact when ISG15-specific siRNA is used to reduce ISG15 activity in IFN-treated cells [31]. This suppression takes place via changing the ubiquitination and protein interaction processes that are necessary for the release of the virus. Ectopic UBEL1 or UBEL1 and ISG15 prohibit the ubiquitination process of Tsg101 and HIV-1-encoded Gag polypeptide and disrupt the association between Tsg101 and the Gag polyprotein's p6 region [31].

ISG15's Impact on Influenza Infection

ISG15 and/or its conjugates are crucial in preventing viruses, namely the influenza A virus, from infecting cells [43]. Since the influenza B virus's NS1 protein (NS1B) was identified to collaborate with ISG15 and restrict it from conjugating target proteins, it has been speculated that ISG15 and/or its linkages are antagonistic to the influenza B virus replication process [43]. The crucial virus-associated protein NS1A is comprised of up of the N-terminal RNA recognition motif (RRM, 1–73 residues) and the effector motif (74–end residues) [44, 45]. In addition to binding double-stranded RNA (dsRNA) [46, 47], the NS1A protein's RBD domain also has a nuclearlocalization signal (NLS) that interacts with importin-α [48]. The RBD's crucial lysine residue (K41) serves as the primary ISG15 attachment point. Herc5, the primary ISG15 E3 enzyme in *Homo sapiens* cells, is directly and specifically bound by the NS1A protein [45]. This modification of ISG15 prevents the RBD from adhering to importin-α while

maintaining the ability of dsRNA to bind [45]. The modification of K41 by ISG15 hinders the replication of the influenza A virus, hence enhancing the antiviral effect of IFN-β [45].

Bacterial Infections

Enteric infections can be caused by the foodborne bacteria Listeria monocytogenes, like other pathogenic bacteria, listeria frequently targets post-translational changes when an infection occurs [49]. ISG15 induction mediated by Listeria is contingent upon the CSP, which detects bacterial genome and transmits signals via STING, TBK1, IRF3, and IRF7 [49]. Nonphagocytic cells, which can generate ISG15 early on, are the first cells to meet Listeria. This could potentially alert innate immune cells regarding the infected site and trigger alarms against an invasive pathogen. An initial unidentified mechanism by which the host activates the immune system's reaction to the diseases caused by viruses and bacteria is the rise in the production of cytokines controlled by ISG15 [49]. IL-6 has been frequently identified as a vital facilitator of the innate immune response to Listeria, while IL-8 is a major mediator of neutrophil and monocyte recruitment in vivo [50, 51].

During M. tuberculosis infestation, one of the premier strongly activated genes by interferons is ISG15. It binds to target substrate (ISGylation), interacts noncovalently with endocellular proteins, and then exits the cell [52]. ISG15 functions as an extracellular cytokine and induces the synthesis of IFN-γ, as supported by studies[53]. It is widely known that IFN-γ plays a crucial activity in the host's ability to resist endocellular bacterial infections, such as M. tuberculosis, and that in both humans and animals, IFN-γ signaling inadequacies significantly raise the risk of acquiring mycobacterial infections [52, 54]. This offers fascinating insights into the capability of ISG15 in the regulation of human mycobacterial diseases [53]

***ISG15 in Defense Against* Pseudomonas Aeruginosa**

ISG15 has a significant impact in corneal innate immune defense opposed to *Pseudomonas aeruginosa* keratitis [55]. The cornea has remarkable resistance to infection under normal circumstances. But pathogenic organisms like *Pseudomonas aeruginosa* can penetrate the epithelial barrier and eventually invade the stroma, causing infectious keratitis. This can happen when immune function is weakened, as it is in diabetic patients, or when wearing contact lenses is frequent [55, 56]. Flagellin pretreatment significantly increases ISG15, which is triggered by *P. aeruginosa* and suggests that the gene may have a defensive function. According to a research study, type 1 IFN is required for isg15 expression in corneas infected with *P. aeruginosa*. In addition to significantly raising corneas' sensitivity to *P. aeruginosa* infection, isg15 reduction also dramatically raised the secretion of IL-1β caused by infection, abolished IFNγ, and decreased CXCL10 expression [55]. Conversely, in Ifnar−/− mice, exogenous ISG15 protects the corneas against and inhibits the growth of *P. aeruginosa* keratitis. The capacity of ISG15 to stimulate antimicrobial peptide production and bactericidal/bacteriostatic action in the cornea is associated with its potential to improve an innate immune response in oppose to *P. aeruginosa* [55]. ISG15 may function in a process associated with CXCL10 and TSLP expression, and dependent on LFA-1 signaling. As an adjectival treatment, ISG15 can strengthen innate mucosal protective immunity against a variety of infections [55].

Cancer

Current studies reveal a considerable rise in the ISG15 pathway, which suppresses the ubiquitin pathway, in several human cancer types [57]. This implies that it might be involved in cancer cells' malfunctioning protein degradation systems. Notably, it has been discovered that human malignant breast cells and clinical samples overexpress the ISG15 pathway, which includes ISG15 and its related enzymes and prevents the degradation of proteins within cancer cells [57]. Specifically, heightened ISGylation obstructs topoisomerase I degradation, crucial for cancer cell survival, in response to camptothecin treatment, thus enhancing the effectiveness of this chemotherapy drug [57]. Additionally, elevated ISGylation impedes protein polyubiquitylation and their turnover in tumor cells, promoting their proliferation [57]. Experimental studies employing gene silencing techniques demonstrated that

ISGylation interferes with proteasome-mediated breakdown of tumor supportive proteins, such as NFAT5 and S100A4, facilitating the transformation of breast cells into cancerous ones. This evince ISG15 fosters tumorigenesis by impeding ubiquitin-mediated protein breakdown, thereby preserving oncogenic proteins [58]. Consistent with this, ISG15 and UBCH8, unique to ISG15 conjugation, stimulate the migration of breast cancer cells by compromising the cell's structural framework and adhesions [58]. Investigations have also revealed regulatory relationship between Ki-Ras and ISG15 in breast-carcinoma cells, where Ki-Ras triggers the ISG15 pathway's expression and ISG15 regulates Ki-Ras by preventing its breakdown via lysosomes [57, 58]. Silencing Ki-Ras or ISG15 pathway normalizes cellular architecture of breast cancer cells. Additionally, elevated levels of ISG15 are linked to aggressive features of breast carcinoma and poor patient outcomes [57, 58]. Beyond breast cancer, ISG15 has been implicated in promoting malignant behaviors in esophageal squamous cells and hepatocellular carcinoma (HCC) cells [59]. In HCC, ISG15 maintains the stability of survivin by sequestering another protein called XIAP, thereby enhancing cancer cell proliferation and migration. Targeting ISG15 with small interfering RNA (siRNA) reduces tumor development in xenograft models of HCC and prolongs the survival of tumor-bearing animals [57, 59]. In addition, literature indicates that extracellular ISG15 slows down tumor formation and promotes the invasion of natural killer (NK) cells into tumors in mouse models [60], while, intracellular ISG15 enhances the surface activity of MHC Class I complexes on breast cancer cells, potentially aiding immune recognition and destruction of cancer cells [60]. These findings suggest that boosting systemic amount of free ISG15 by targeting enzymes involved in its conjugation may hold promise as a therapeutic strategy for cancer patients [60].

Neurodegenerative Disorders

Recent research has underscored the potential significance of ISG15 in defending against neurodegenerative disorders, making it a subject of considerable interest in the scientific community [61]. ISGylation involves the attachment of ISG15 to residues of lysine on substrate proteins and is mediated by a series of enzymes. This process regulates mitophagy and autophagy, essential pathways for removing damaged organelles and proteins. These mechanisms prevent the accumulation of toxic aggregates associated with neurodegenerative diseases [62, 63]. Studies specify an increase in ISG15 expression in the cortical and deep gray matter of multiple sclerosis (MS) patients, suggesting a connection between ISG15 and neuroinflammation. It has been demonstrated that ISG15 CD11b-dependently activates microglia, which may have critical function in the inflammatory seen in neurodegenerative disorders [64].

Furthermore, ISG15 governs mitochondrial function, which is vital for neuronal health. It regulates oxidative phosphorylation (OXPHOS), ATP production, and reactive oxygen species (ROS) levels, all the processes are essential for meeting neuronal energy demands and preventing oxidative damage [62]. Increased ISG15 expression and ISGylation during demyelination in various regions of the nervous system suggest protective role of ISG15. This notion is supported by findings in IFNAR knockout mice, which exhibit reduced ISGylation in the central nervous system during models of demyelination [61–63]. More specifically, IFNy-treated neurons indicated increased ISGylation and ISG15, suggesting its protective mechanism. Ongoing research aimed at identifying ISG15 targets in human neurons may shed information on functions of ISGylation in neurodegenerative diseases and potentially lead to novel therapeutic strategies for conditions like MS [64]. ISG15 functions, from regulating mitochondria to modulating immune responses, are crucial for developing new approaches to combat neurodegenerative diseases. As research progresses, the significance of ISG15 in neuronal health and disease continues to emerge, highlighting its potential as a key player in neuroprotection [61, 64].

In conclusion, ISG15 and the process of ISGylation are essential for defending against neurodegenerative disorders by regulating mitochondrial function, immune cell activation, and potentially preventing the accumulation of toxic protein aggregates [64]. In-depth research is essential to comprehend ISG15's intricate roles in the nervous system and its therapeutic potential.

COUNTERMEASURES TO REVERSE ISGYLATION BY VIRUSES: DEISGYLATION ACTIVITY

Deconjugation of ISG15

The primary Deubiquitinases (DUB) that reverses ISGylation is ubiquitin-specific protease 18 (USP18), also known as UBP43 and have molar mass 43 kDa. Originally detected in acute myeloid leukemia 1 (AML1)-eight twenty-one (ETO) knock-in mice [12, 65], it was subsequently recognized in the research study of human melanoma cell lines [66] and virus-infected porcine alveolar macrophages [67]. USP18 has been recognized as versatile protein, even without catalytic activity it suppresses type I IFN activity [12]. As a deconjugating enzyme, it also annuls ubiquitination or ISGylation, further breaking down the code produced by these reactions. Thus, it has been proposed that USP18 is engaged in a varied cellular activity, including signaling, responses to stress, bacterial and viral infections [68, 69].

ISG15 gets stimulated by IFN-α/β, leading to its upregulation and conjugation with numerous proteins via a tripartite enzymatic cycle [70, 71]. IFN-inducible cysteine protease USP18's isopeptidase active site regulates the ISG15 deconjugation process. This domain features adjacent asparagine, a histidine box containing histidine, and cysteine box including cysteine. These sequences are required for USP18's enzymatic function [71, 72]. Using 125I-labelled ubiquitin and Ubls to analyze USP18's selectivity with ISG15, SUMO, and NEDD8, it has been determined that USP18 selectively eliminates ISG15 from its associated protein targets [73]. USP18 can be stabilized by ISG15 on its own; this stability avoids the undesired autoinflammatory impact of prolonged IFN-α/β (71,74). Besides ISG15, USP18 also selectively prevents K63-linked ubiquitination of NEMO, which results in the TAK1-TAB complex-induced negative control of NF-κB activation [71, 75].

Viruses Have Developed Strategies to Annul ISGylation

To counteract host reactions, several viruses, particularly those belonging to the order Nidovirales, encompassing coronaviruses, express enzymes that can deconjugate ubiquitin and ISG15 from target proteins. OTU-containing proteases are found in L protein that is encoded by the Crimean-Congo Hemorrhagic Fever virus (CCHFV), Porcine reproductive and respiratory syndrome virus (PPRSV), also including Alpha-arterivirus equid, that cause horse arteritis. It has been demonstrated that these proteins lower cellular ISG15 and ubiquitin conjugates [13, 76]. The PLpro that are encoded by coronaviruses, such as the human coronavirus-NL63, Hepatoencephalitis virus strain 3, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV), also deubiquitylate and deISGylate target proteins [13, 77, 78].

ISG15 conjugation inhibits the viral gene functioning and virion release, prohibiting human cytomegalovirus (HCMV) growth [13]. Several countermeasures have been developed by HCMV to overcome this. By blocking STAT2 and ISG promoter binding, the main immediate-early protein IE1 suppresses ISG transcription. ISG15 protein conjugation was inhibited by ectopic expression of HCMV IE1, most likely because of HERC5 and other ISG15 conjugation machinery components being expressed less [13, 79]. Furthermore, ISG15, UBE1L, and HERC5 non-covalently bind p21 and p27 (pUL26 genes products) important in virion integrity and suppression of NF-κB signaling [13, 80]. When ISG15 and the conjugating enzymes were co-transmitted into cells, UL26–p21 expression lowered ISG15 conjugates levels [13].

CONCLUSION

ISGylation emerges as a versatile and indispensable defence mechanism in the host's inherent immunological response to viral infections. It crucially modulates immunity and suppresses viral replication. The reversibility of ISGylation, coupled with structural insights into its molecular mechanisms, underscores its significance in finely tuning the immune response. Moreover, the identification of ISGylation substrates and its clinical implications highlight its potential as a target for therapy for various diseases. Continued research into the intricacies of ISGylation promises to unveil

new insights into host-pathogen interactions and pave the way for innovative strategies to combat viral infections and associated diseases.

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